Ano5 Cas9-KO Strategy

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Design Date: 2019-9-19
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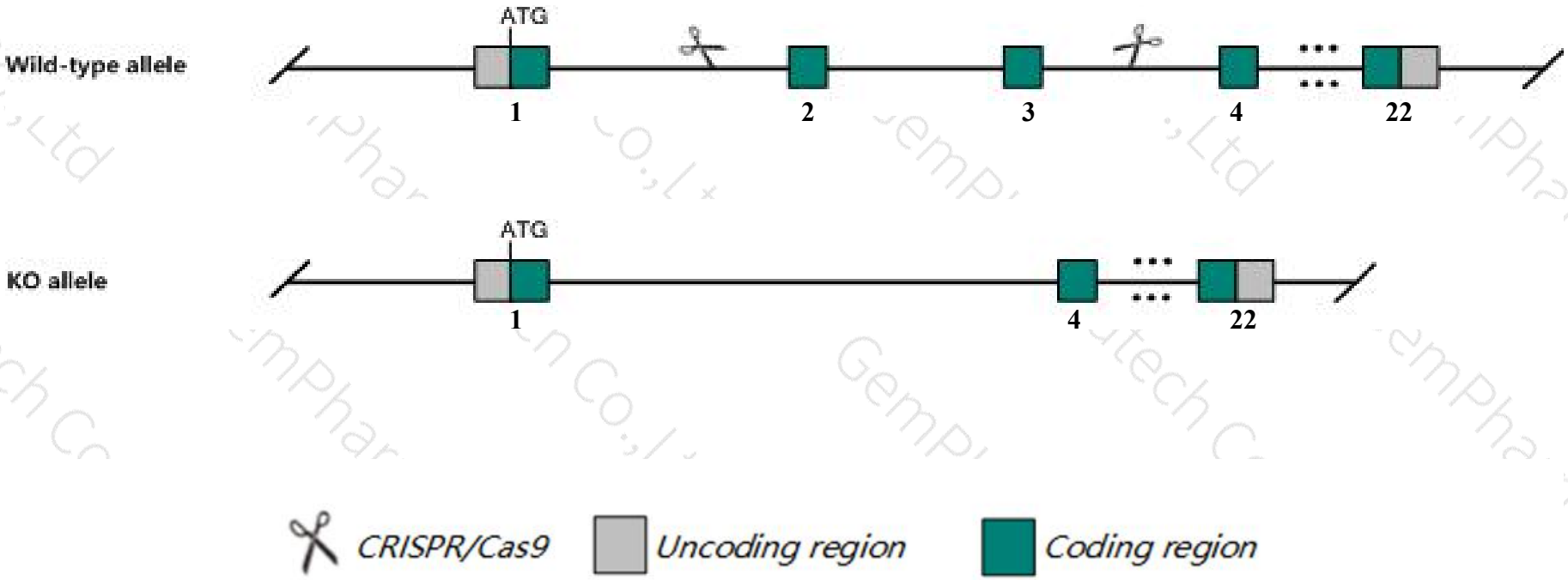
Project Overview

Project Name	***Ano5***
Project type	**Cas9-KO**
Strain background	**C57BL/6JGpt**

Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ano5* gene. The schematic diagram is as follows:



Technical routes

➢ The *Ano5* gene has 3 transcripts. According to the structure of *Ano5* gene, exon2-exon3 of *Ano5-201* (ENSMUST00000043944.5) transcript is recommended as the knockout region.The region contains 98bp coding sequence. Knock out the region will result in disruption of protein function.

➢ In this project we use CRISPR/Cas9 technology to modify *Ano5* gene. The brief process is as follows: CRISPR/Cas9 system

Notice

- According to the existing MGI data,One type of homozygous KO causes abnormalities in skeletal muscle mitochondria and impairs muscle regeneration and repair, leading to exercise intolerance. Another type of homozygous KO impairs sperm motility, leading to male subfertility.
- The strategy knockout region contains the initial codon of Ano5-202, there is a risk that a new ATG-encoded unknown protein will be formed after knockout.

- The *Ano5* gene is located on the Chr7. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.

- This Strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of the gene knockout on gene transcription, RNA splicing and protein translation cannot be predicted at the existing technology level.

Gene information（NCBI）

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Ano5 anoctamin 5 [Mus musculus (house mouse)]

Gene ID: 233246, updated on 31-Jan-2019

Summary

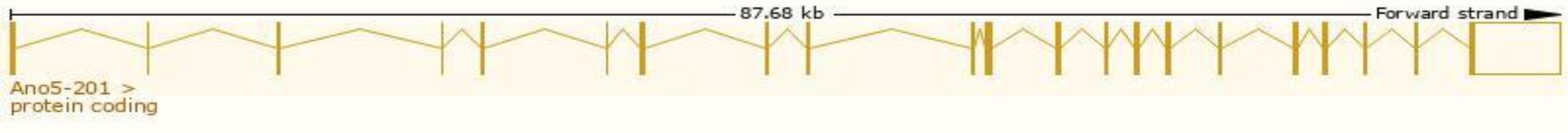
Official Symbol	Ano5 provided by MGI
Official Full Name	anoctamin 5 provided by MGI
Primary source	MGI:MGI:3576659
See related	Ensembl:ENSMUSG00000055489
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	9330162L24, Gdd1, Tmem16e
Summary	This gene encodes a member of the anoctamin family, which in mammals is comprised of 10 members. Anoctamin proteins are proposed to have eight transmembrane domains with both termini facing the cytoplasm and a C-terminal domain of unknown function. While some members have been characterized as calcium-activated chloride channels, this protein is reported to have little anion conductance activity. Elevated levels of this protein were found in dystrophic mice. In humans, mutations of this gene are associated with with musculoskeletal disorders such as myopathies, muscular dystrophy and gnathodiaphyseal dysplasia. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2012]
Expression	Low expression observed in reference dataset See more
Orthologs	human all

Transcript information（Ensembl）

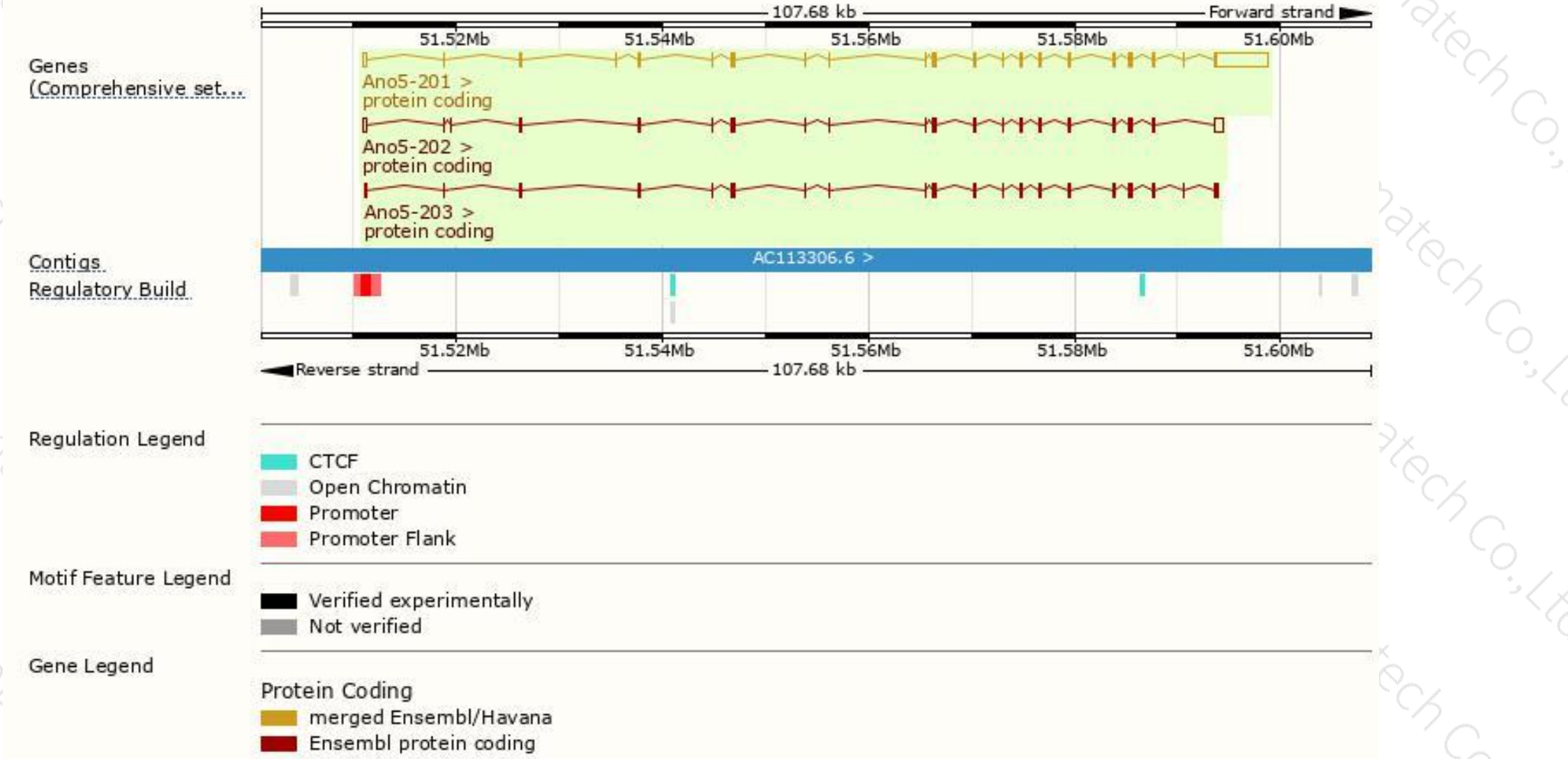
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ano5-201	ENSMUST00000043944.5	7785	904aa	Protein coding	CCDS39968	Q75UR0	TSL:1 GENCODE basic
Ano5-203	ENSMUST00000207717.1	2753	854aa	Protein coding	CCDS85311	Q75UR0	TSL:1 GENCODE basic APPRIS P1
Ano5-202	ENSMUST00000207044.1	3311	772aa	Protein coding	-	Q75UR0	TSL:1 GENCODE basic

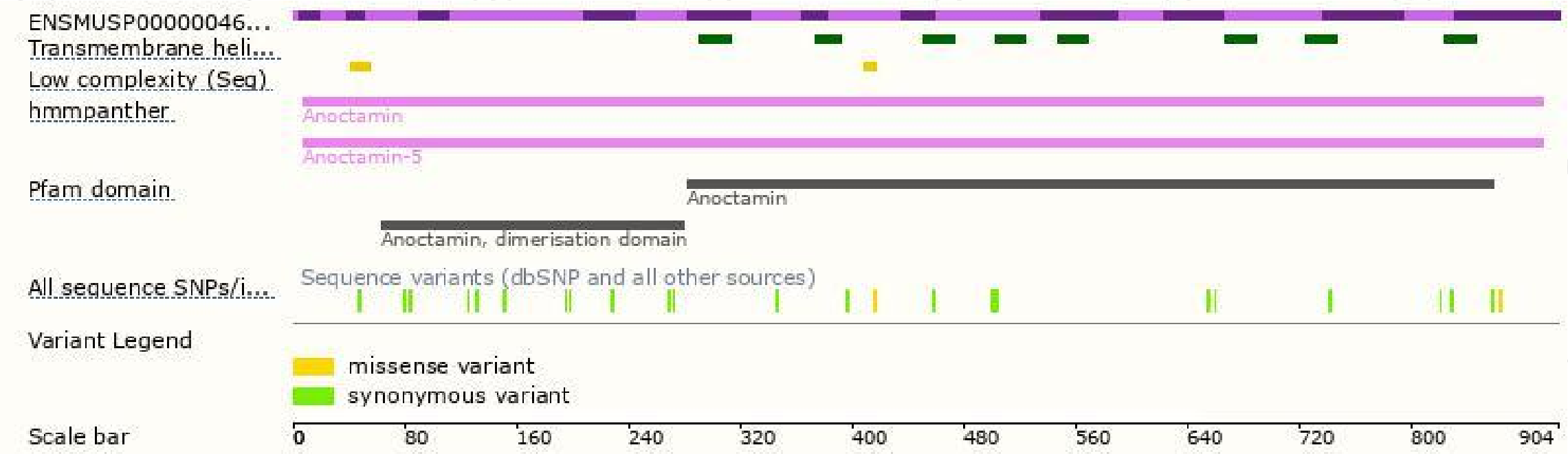
The strategy is based on the design of *Ano5-201* transcript,The transcription is shown below



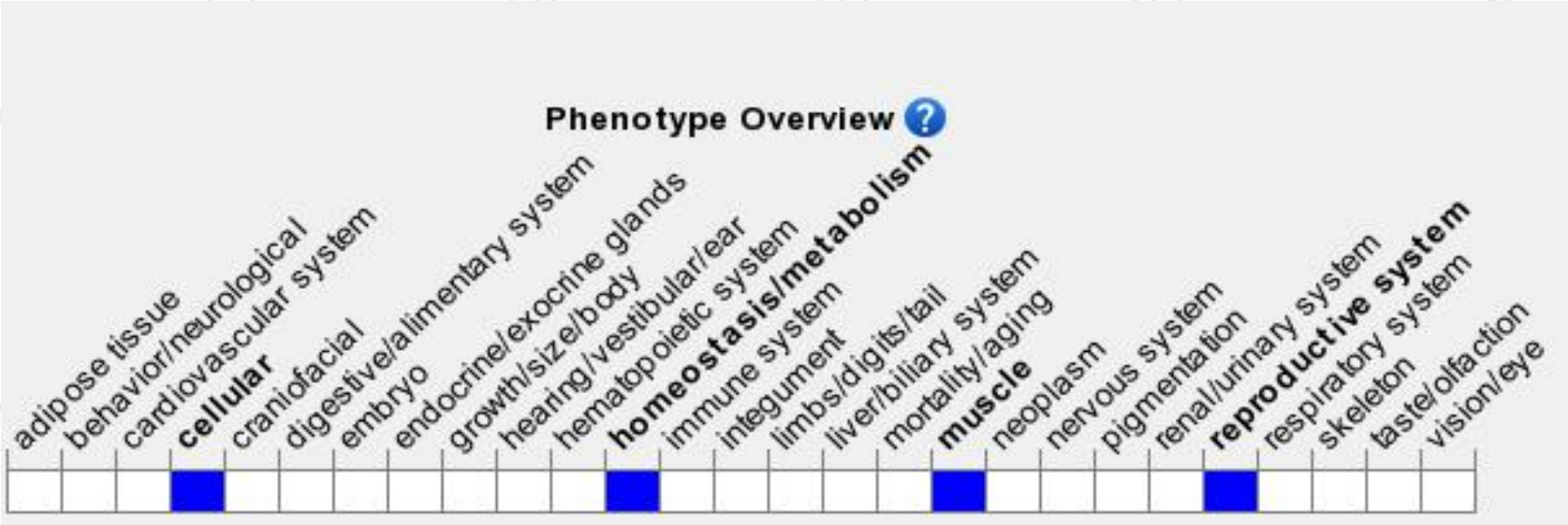
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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If you have any questions, you are welcome to inquire.

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